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14. ABSTRACT

In this final report, we show gene therapy using re-engineered super p53 (p53-CC constructs) kills some ovarian cancer cell lines in vitro, but was not superior to wild-type p53, and showed toxicity in normal cells. Combination therapy using gene therapy and chemotherapy proved to be difficult, due to the genel transfection inhibition by paclitaxel. In order to overcome these issues, a mitochondrially targeted p53 (p53-MTS) was used, and was found to be more potent in killing 2 different ovarian cancer cell lines (superior to wt p53, and p53-CC). From this, p53-MTS will then serve as a lead construct. Technical skills gained in the proposal include cell culture, transfections, microscopy, apoptosis assays, transcriptional assays, polymer synthesis, cloning, adenoviral vector preparation, and in vivo work. Importantly, we have verified the ovarian cancer animal model and are able to create ovarian tumors in mice. Our polymer-adenovirus constructs were optimized in vitro and in vivo, and did not show gross signs of toxicity. Future studies ongoing in the lab include optimization of cancer-specific promoters (to drive production of p53-MTS in cancer cells only), and testing polymer-adenovirus encoding p53-MTS in vivo, which are expected to reduce ovarian cancer tumors.

15. SUBJECT TERMS

Ovarian cancer, gene therapy, p53, modified p53, tumor suppressor, high grade serous carcinoma, combination therapy, carboplatin, paclitaxel, polymeric drug delivery, polymer-adenovirus hybrid

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1. **INTRODUCTION:**
   Subject: This project used a re-engineered p53 (called super p53) for gene therapy that is not subject to dominant negative inhibition by mutated p53 in cancer cells. This super p53 is modified to contain a segment called a coiled-coil domain that allows binding with itself only (resulting in auto-activation). Super p53 cannot bind to other proteins in cancer cells, including wt p53, and subsequently is not inactivated. Purpose: use super p53 for gene therapy of ovarian cancer to allow tumor suppressor/anti-cancer activity. Scope: this super p53 gene therapy can be used in ovarian cancer patients regardless of their p53 status or other genetic heterogeneity. In the proposal and in this report, super p53 = p53-CC (first generation construct) or p53-CCmut (2nd generation construct; also referred to as p53-CC* in some graphs). Note: after inferiority of p53-CC, a mitochondrially targeted p53 (p53-MTS) was tested, and was found to be superior.
2. **KEYWORDS:**
   Ovarian cancer, gene therapy, p53, modified p53, tumor suppressor, high grade serous carcinoma, combination therapy, carboplatin, paclitaxel, polymeric drug delivery, polymer-adenovirus delivery
3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Specific Aim 1: Determine if a re-engineered, super p53 (p53-CCmut) is capable of tumor suppressor activity (measured by apoptosis, proliferation, transformative ability and gene expression profiling) and can bypass the dominant negative effect in ovarian cancer cell lines (with varying p53 status) alone, and in combination with standard of care, carboplatin and paclitaxel (CPTX).	**Timeline**	**Percent Completion**	**Site 1**	**Site 2**
Major Task 1: Test activity of p53-CCmut with or without CPTX in ovarian cancer cell lines	Months			
Subtask 1: Test apoptotic activity of p53-CCmut with or without CPTX (caspase 3/7, Annexin V, DNA segmentation, 7-AAD, TUNEL) in cells with varying p53 status. Cell lines used: Kuramochi Caov-4, OVCAR-3, OVCAR4, SKOV3, SKOV3ip, normal cells: BJ, IHOEC	1-8	20% completed; halted due to interference of transfection by paclitaxel and due to poor performance of p53-CC; will continue	Dr. Lim	

		with p53-MTS		
Subtask 2: Test ability of p53-CCmut with or without CPTX to inhibit cell proliferation and oncogenic potential (trypan blue, MTT, CFA) in cells with varying p53 status. (Same cell lines as subtask 1)	8-10	5% completed; same continuation as above	Dr. Lim	
Subtask 3: Measure p53 gene expression profile of p53-CCmut with or without CPTX Cell line used: SKOV3	10-12	0% (will be continuing with p53-MTS)	Dr. Lim	
Milestone(s) Achieved: p53-CCmut is capable of killing all ovarian cancer cell lines tested; does not kill normal cells; p53-CCmut activates same genes as wt p53	12		Dr. Lim	Dr. Janát-Amsbury
Local IACUC Approval	3	100%	Dr. Lim	Dr. Janát-Amsbury
Submission of institution approval of animal protocol and related materials for DOD's ACURO approval	3	100%	Dr. Lim	Dr. Janát-Amsbury
Receive ACURO approval before initiating animal experiments	complete	100%	(DOD)	
Specific Aim 2: Deliver super p53 DNA with advanced polymeric systems alone and in combination with CPTX first *in vitro*, then *in vivo* by intraperitoneal injection into a syngeneic orthotopic metastatic mouse ovarian cancer model [33]. 2 polymeric delivery systems will be tested: a. Water soluble lipopolymer (PEG-PEI-cholesterol) [45] currently being used in clinical trials [24, 46] b. RGD-conjugated bioreducible polymer-coated adenovirus (CD-PEG-RGD) The delivery method that provides the highest expression of the gene and highest cell-killing activity *in vitro* will proceed to the *in vivo* testing phase.				
Major Task 2: in vitro testing with polymers				
Subtask 1: deliver p53-CCmut plasmid with PEG-PEI-cholesterol polymer to ID8	13-16	100% completion-	Dr. Lim	Dr. Janát-Amsbury

ovarian cancer cells; measure transfection efficiency and potency Cell line used: ID8.		WSLP has been synthesized, low transfection efficiency		
Subtask 2: construct adenoviral vector containing p53-CCmut; deliver p53-CCmut in adenovirus with CD-PEG-RGD) polymer to ID8 ovarian cancer cells; measure transfection efficiency and potency	13-19	80% completion with control Ad only; ongoing with p53-MTS	Dr. Lim	Dr. Janát-Amsbury
Milestone(s) Achieved: Determination of optimal polymeric system for in vivo studies	19		Dr. Lim	Dr. Janát-Amsbury
Major Task 3: in vivo testing with optimal polymer				
Subtask 1: establishing primary tumor within ovary	19-21	100% completion (able to generate ovarian tumors)		Dr. Janát-Amsbury
Subtask 2: testing of p53-CCmut using optimal polymeric system with and without CPTX	21-24	10% completed (tested control Ad only)	Dr. Lim	Dr. Janát-Amsbury
Milestone(s) Achieved: tumor reduction and reduced metastasis with p53-CCmut (with or without CPTX; completion of 1-2 manuscripts (in vitro and in vivo)	24	Ongoing with p53-MTS constructs	Dr. Lim	Dr. Janát-Amsbury

- **What was accomplished under these goals?**

Aim 1, Subtask 1: Test apoptotic activity of p53-CCmut with or without CPTX (caspase 3/7, Annexin V, DNA segmentation, 7-AAD, TUNEL) in cells with varying p53 status.

Major activities, specific objectives, results:

Cell Culture: major activities and specific objectives are to obtain, culture, and propagate cell lines The cell lines successfully cultured included ovarian cancer cell lines:

-Kuramochi (high grade serous carcinoma-HGSC dominant negative p53 with D281Y mutation)

-OVCAR-3 (HGSC, dominant negative p53 with R248Q gain-of-function mutation)

-SKOV3 (derived from ascites, p53 null)

-SKOV3ip1 (more aggressive version of SKOV3, p53 null)- same culture and transfection conditions as SKOV3.

-BJ normal cells

For culture and transfection conditions, see forthcoming data.
Cell lines not used, and reasons why:
-Caov-4 (HGSC derived from metastatic site, V147D p53 mutant) ovarian cancer cells require growth without CO_2 and specialized media; we were not able to grow and transfect these cells.
-OVCAR-4 (HGSG, L130V mutant, platinum resistant) cells were not used due to negative results in other cell lines (see forthcoming data).
-IHOEC cells were not used, due to results in BJ normal cells. In Table 1 below (taken from the original proposal), yellow highlight = cells successfully grown and propagated in culture

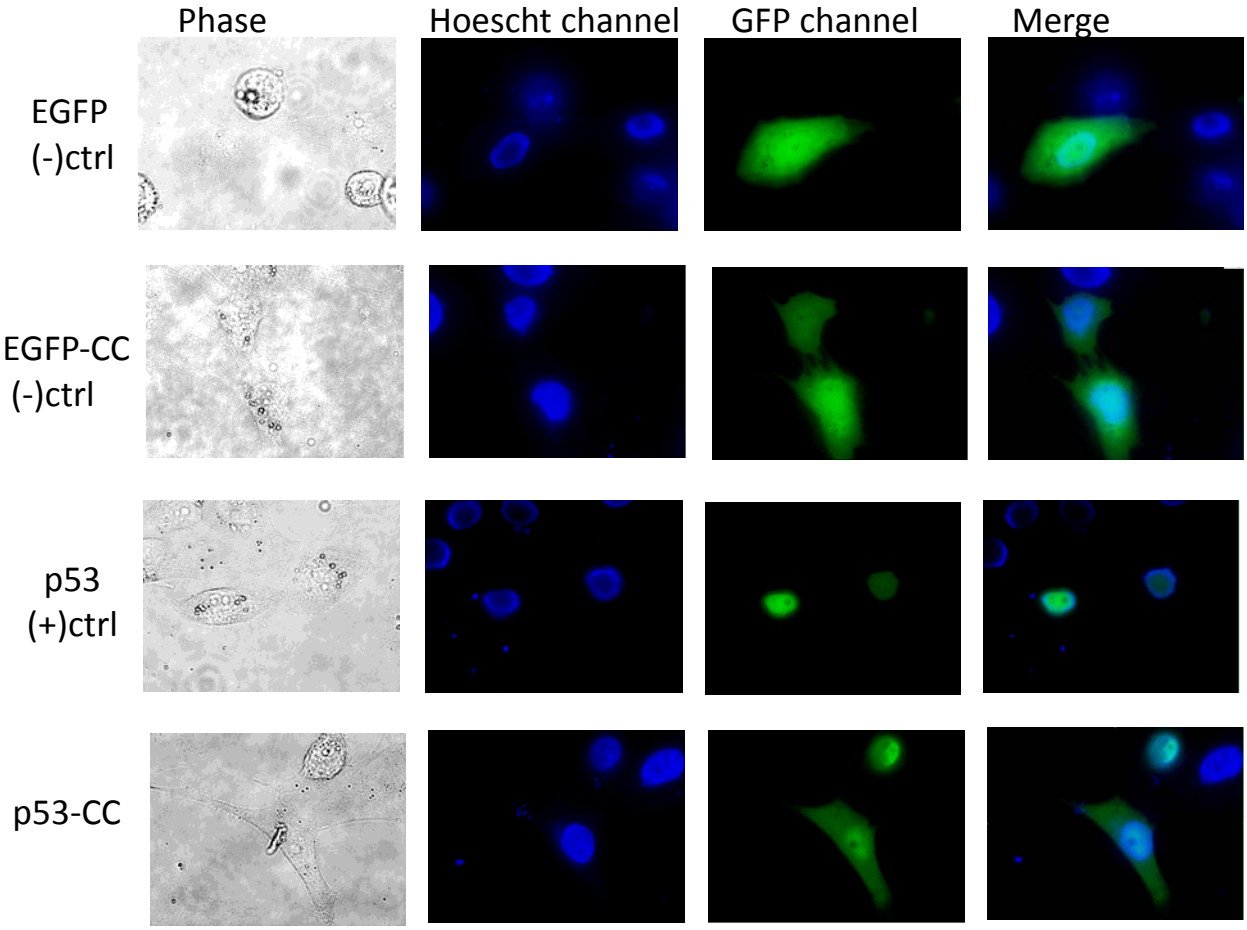
Table 1. Ovarian cancer cells:	**p53 status**	**BRCA1/2 status**	**Characteristics (all cell lines are human except ID8)** (note: all cell lines are commercially available)
Kuramochi	Dom neg,D281Y	BRCA1 mut	From ovarian cancer ascites; epithelial-like morphology, HGSC
Caov-4	V147D mut	Wt	Ovarian adenocarcinoma; derived from metastatic site (fallopian tube); likely to be HGSC
OVCAR-3	Dom neg, gain of fct, R248Q	Wt	Ovarian adenocarcinoma; epithelial; HGSC
OVCAR-4	L130V mut	Wt	Serous ovarian adenocarcinoma; HGSC; *resistant to platinum*
SKOV3	p53 null	Wt	Ovarian adenocarcinoma; from ascites; not likely to be HGSC
SKOV3.ip1	p53 null	Wt	Ovarian adenocarcinoma; more aggressive version of SKOV3
ID8 cells	p53 null	Wt	Murine ovarian surface epithelial cells spontaneously transformed
Normal cells: BJ, IHOEC	Wt p53	n/a	BJ: Normal fibroblasts; IHOEC: SV40 immortalized ovarian epithelial cells

Subcloning and expression of plasmids: major activities and specific objectives are to successfully subclone, express in *e. coli*, and purify (by maxiprep) plasmids. All plasmids used for this proposal have been made and propagated including:

-p53-CC
-p53-CCmut (p53-CCmutE34K-R55E)
-pEGFP (negative control)
-pEGFP-CC (negative control)
-wt p53 plasmid (positive control in some cases; used for comparison to p53-CC and p53-CCmut constructs.

Transfection optimization of cell lines and initial testing by fluorescence microscopy (to visualize transfected and expressed constructs, and to check for proper subcellular localization): major activities and specific objectives are to optimize transfection conditions and evaluate time points (24-72h) where expression of constructs can be visualized and verified via fluorescence microscopy. Kuramochi (80,000 cells per 4 well chamber) or ID8 cells (40,000 cells per 4 well

chamber) were seeded, and 24h later, transfected with 1.25ug plasmid DNA and 1.25ul Lipofectamine 2000. Kuramochi cells were imaged 24h post-transfection, while ID8 cells wereimaged 16h post-transfection. For Hoescht staining, 2ul of 1mg/ml H33342 dye was added to 500ul media/cells, and rinsed with PBS 15min prior to fluorescence microscopy. Results are shown in Figures 1 and 2, microscopy images of various constructs (listed on left side of diagram). In Kuramochi cells (Figure 1), p53 and p53-CC constructs localize mostly in the nucleus (although p53-CC has some cytoplasmic localization). In ID8 cells (Figure 2), p53 and p53-CC localize to

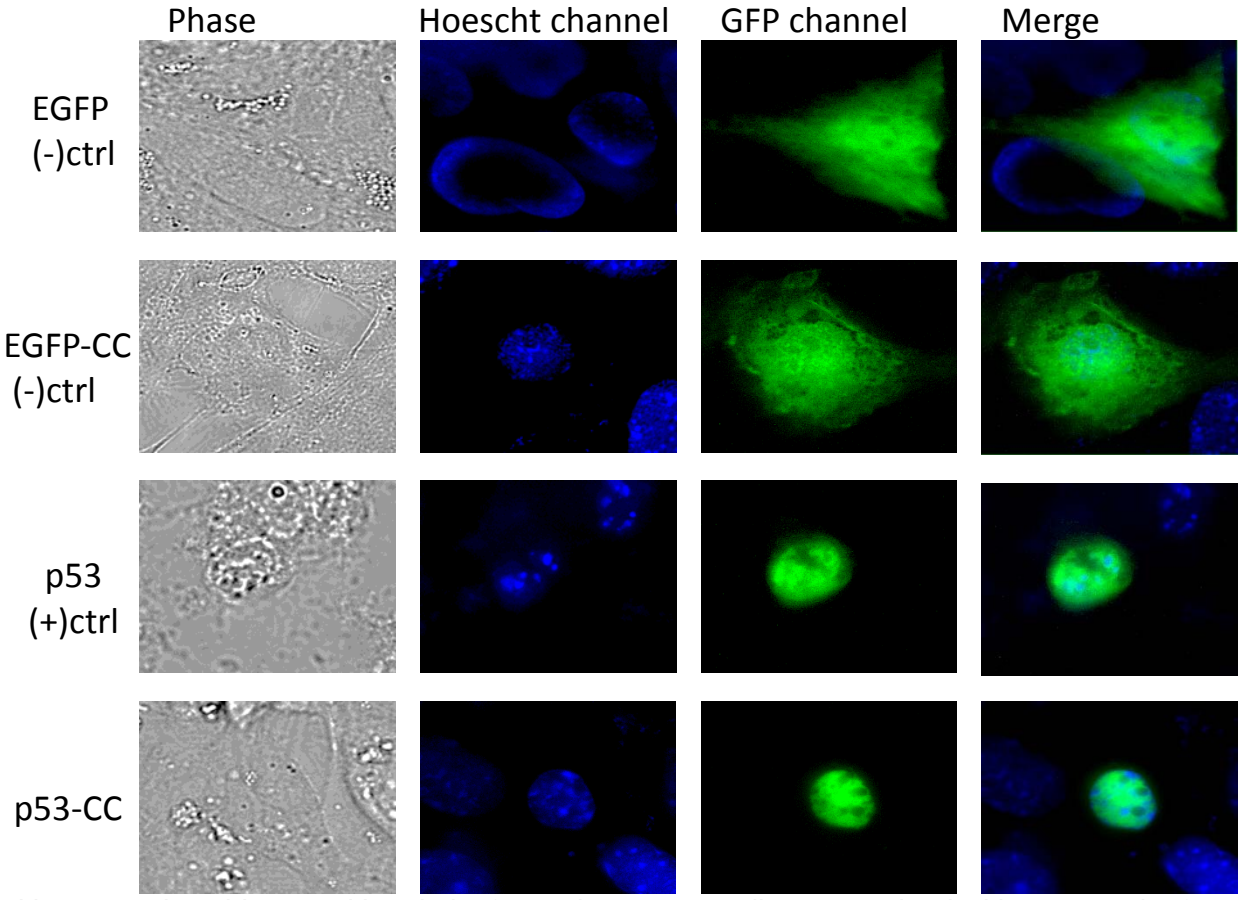


the nucleus as expected. In both cell lines, EGFP and EGFP-CC negative controls localize throughout the cell. In these experiments, we verify that we can transfect 2 cell lines with our plasmid constructs, the proteins are expressed, and localize in the nucleus as expected.

Figure 1. Kuramochi ovarian cancer cells transfected with various constructs (listed on left side). Phase, Hoescht (nuclear staining) channel, GFP channel (all constructs tagged with GFP), and merge panels shown.

Transfection optimization of cell lines and initial testing by measurement of apoptosis (7AAD): major activities and specific objectives are to optimize transfection conditions and evaluate time points (24-72h) where maximal apoptosis of constructs occurs in cell lines measured by 7AAD. Transfections for the 7AAD assay using negative control (EGFP) and positive control (wt p53) have been optimized in Kuramochi, ID8, and OVCAR3 cells. Details are as follows:

Kuramochi cells:
Media: RPMI with 10% heat-inactivated FBS, 1% penicillin/streptomycin/glutamine.
Transfection: 500,000 cells seeded in 6-well plate (for 7AAD), and transfected 24h after seeding



with 5ug plasmid DNA with 5ul Lipofectamine 2000. Cells were stained with 7AAD 48h after transfection, then analyzed and gated for EGFP using the FACSCanto-II/FACSDiva software.

Figure 2. ID8 ovarian cancer cells transfected with various constructs (listed on left side). Phase, Hoescht (nuclear staining) channel, GFP channel (all constructs tagged with GFP), and merge panels shown.

ID8 cells:
Media: DMEM with 5% HI-FBS, 1% penicillin/streptomycin/glutamine, 1% ITSX
Transfection: 125,000 cells seeded in 6-well plate and transfected 24h after seeding with 7.5ug plasmid DNA with 7.5ul Lipofectamine 2000. Cells were stained and analyzed as above.

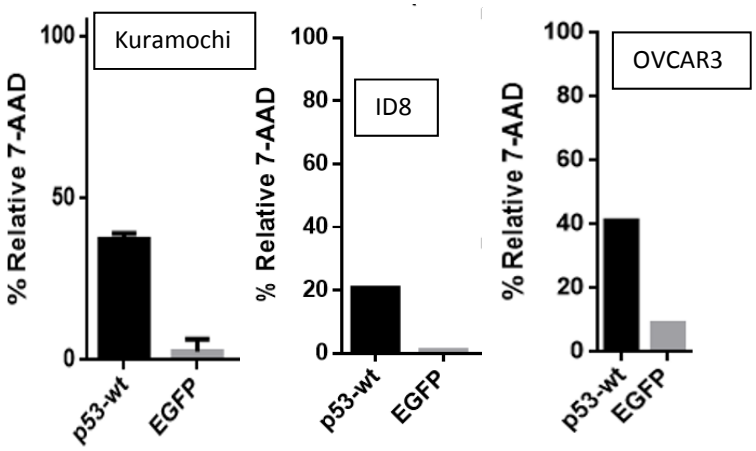
OVCAR3 cells:
Media: RPMI with 20% FBS, 1% penicillin/streptomycin/glutamine, 1% bovine insulin.
Transfection: 250,000 cells seeded in 6 well plate and transfected 24h after seeding with 5ug plasmid DNA and 5ul Lipofectamine 2000. Cells were stained and analyzed as above.

SKOV3 cells:
Media: DMEM with 10% FBS, 1% penicillin/streptomycin/glutamine
Transfection: 200,000 cells/well seeded in 6 well plate and transfected 24h after seeding with 5ug plasmid DNA and 5ul Lipofectamine 2000.

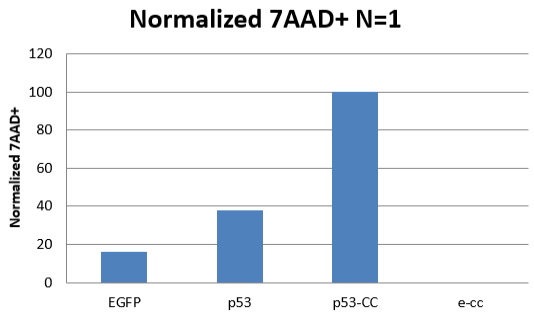
BJ cells:
Media: DMEM with 10% FBS, 1% penicillin/streptomycin/glutamine
Transfection: 250,000 cells/well seeded in 6 well plate and transfected 24h after seeding with 5ug plasmid DNA and 5ul Lipofectamine 2000.

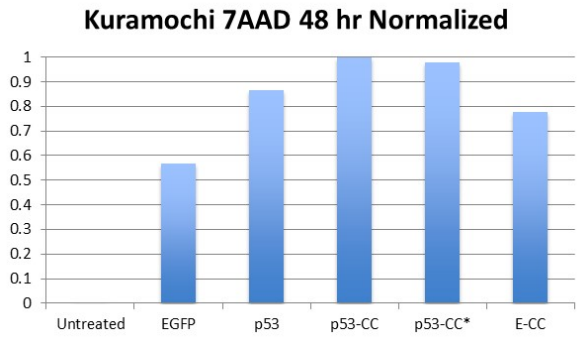
Figure 3 shows preliminary data for 3 cell lines; in all cases, wt p53 shows apoptotic activity, while EGFP control has low activity, as expected.

Figure 3. 7AAD activity plots for Kuramochi, ID8, and OVCAR 3, respectively. Positive control (p53 wild type) and negative control (EGFP) tested so far (n = 3 when error bars shown, otherwise n = 2).



Data for Kuramochi cells with the p53-CC construct is in Figures 4A, also in ID8 cells in Figure 4B. Kuramochi cells proved to be difficult to transfect, with low transfection efficiency, and some cell death occuring from transfections no matter what gene was transfected. We were unable to conclude superiority of p53-CC compared to wt p53. This may be due to the inherent overexpression of proteins that occur with transfection. In Figure 4B, the p53-CCmut (p53-CC*) demonstrates activity in ID8 cells in vitro, but again not superior to wt p53 (which is expected in these cells which do not contain dominant negative p53). Regardless, the effect of p53-CC* was underwhelming, and was not superior to wt p53.





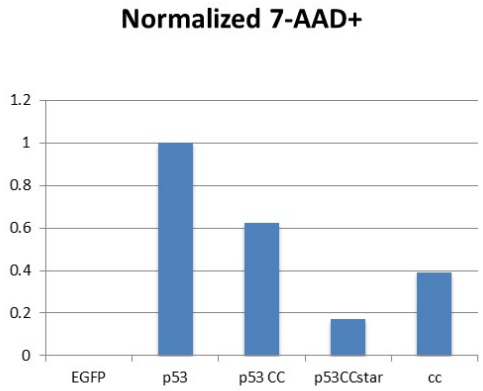
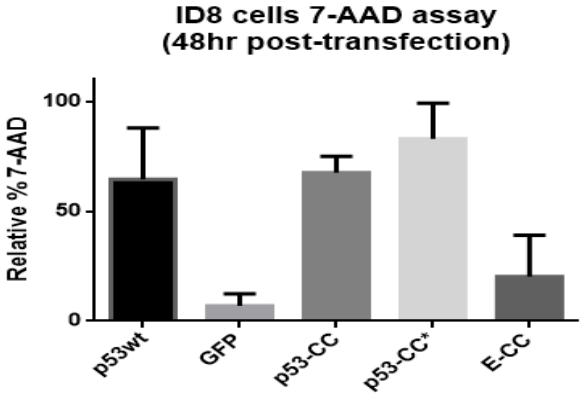


Figure 4A. Apoptotic activity of various constructs in Kuramochi cells, 3 different experiments. p53-CC construct appears to have apoptotic activity (e-cc = EGFP-CC). N=1 unless otherwise indicated.

Figure 4B. Apoptotic activity of various constructs in ID8 cells. p53-CC* (final p53-CCmut construct) appears to have robust apoptotic activity compared to other constructs. (E-CC = EGFP-CC negative control).



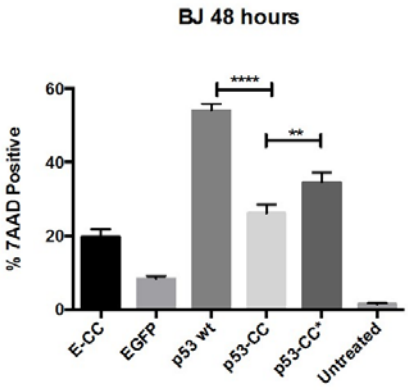


Figure 4C. Testing toxicity of constructs in normal BJ cells. Wt p53, p53-CC, and p53-CC* were all (unexpectedly) active (caused apoptosis) in normal cells. N = 3. ANOVA with Tukey's post- test.

The effect of the p53-CC construct (and others) was also tested in normal BJ cells. We had hypothesized that these constructs would not be active due to the normal degradation of p53 by MDM2 in normal cells. However, as indicated in Fig.

4C, constructs were active, indicating they may have an impact on normal cells.

Due to the disappointing activity of p53-CC or p53-CC*, we sought to find other p53 constructs that were more potent. We therefore tested our previously published mitochondrially targeted p53 constructs (p53-MTS) which have been only used in breast cancer (Matissek, K.J., Okal, A., Mossalam, M., and Lim, C.S. Delivery of a monomeric p53 subdomain with mitochondrial targeting signals from pro-apoptotic Bak or Bax. Pharm Res, 2014 Sept 31; (9):2503-15). Mitochondrially targeted p53 kills cancer cells via a direct induction of apoptosis that does not require activation of hundreds of genes, and overcomes the dominant negative effect since p53-MTS acts as a monomer, and does not need to dimerize for its activity. Although this previous paper focused on shorter versions of p53 attached to a mitochondrial targeting signal, for this DOD proposal, we focused on full length p53-MTS constructs, with MTSs from Bak and Bax mitochondrial proteins since they had more activity that the shorter constructs in ovarian cancer cell lines. **Figure 5** below shows that p53-MTS constructs can colocalize with the mitochondria.

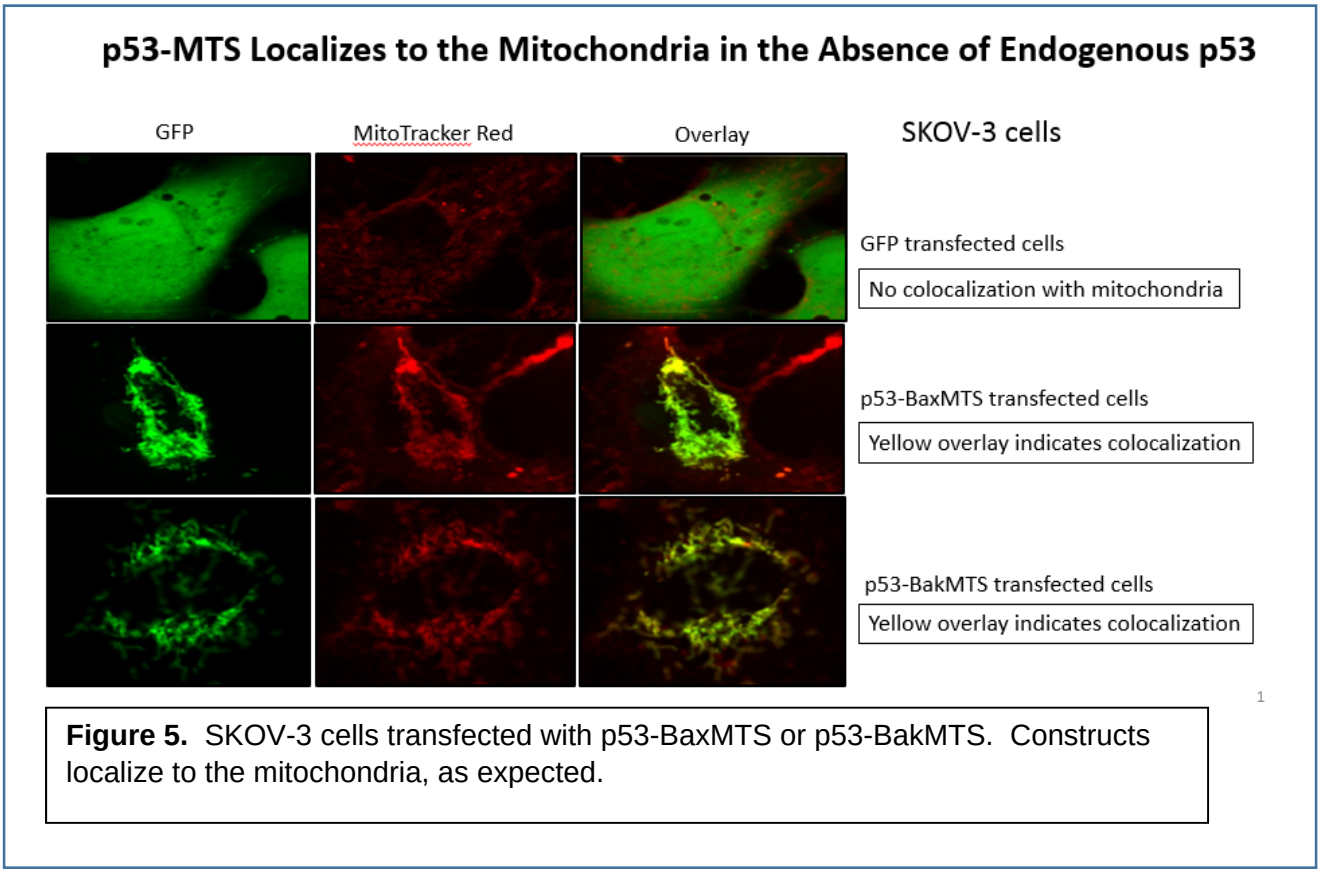
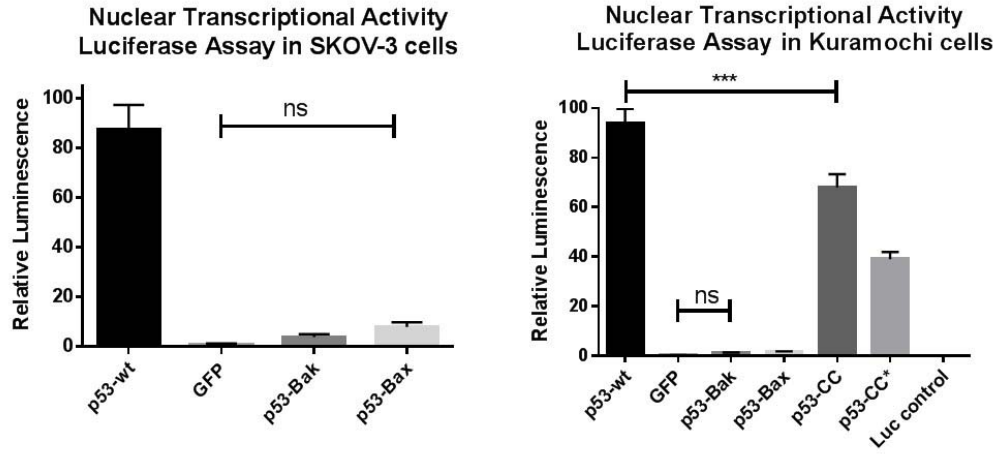


Figure 5. SKOV-3 cells transfected with p53-BaxMTS or p53-BakMTS. Constructs localize to the mitochondria, as expected.

We also tested the p53-MTS constructs for lack of nuclear activity in 2 ovarian cancer cell lines, Fig. 6. The MTS constructs had a lack of nuclear activity, while wt-p53, p53-CC, and p53-CC* constructs had nuclear activity, as expected.

Figure 6. SKOV-3 or Kuramochi cells nuclear transcriptional activity. N = 3; ANOVA with Tukey's post- test.



Finally, p53-MTS constructs were tested for apoptotic activity in 2 cell lines. Caspase assay in ID8 cells, and 7-AAD assay in OVCAR-3 and ID8 cells show superiority of p53-Bak MTS and p53-Bax constructs over wt-p53 and p53-CC as shown in Fig. 7.

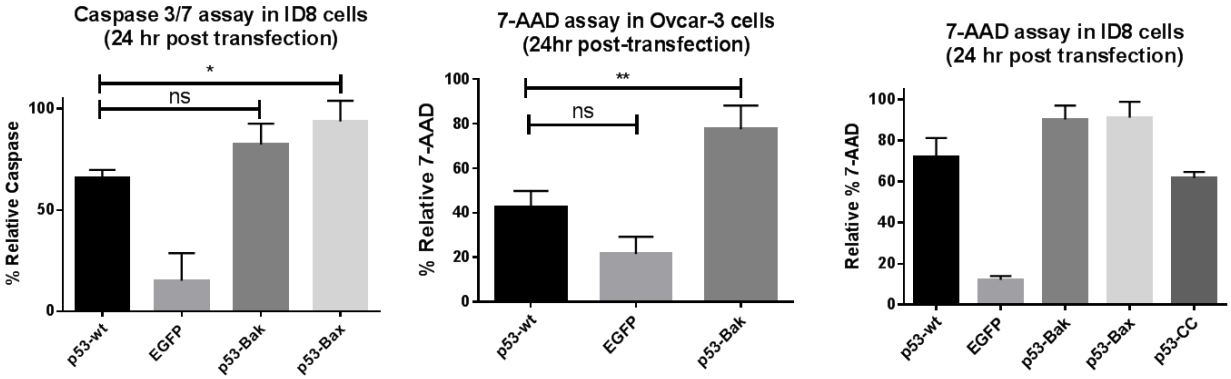


Figure 7. Caspase and 7-AAD assays in ID8 and/or OVCAR-3 cells. (p53-Bak = p53-BakMTS; p53-Bax = p53BaxMTS). N=3; ANOVA with Tukey's post- test.

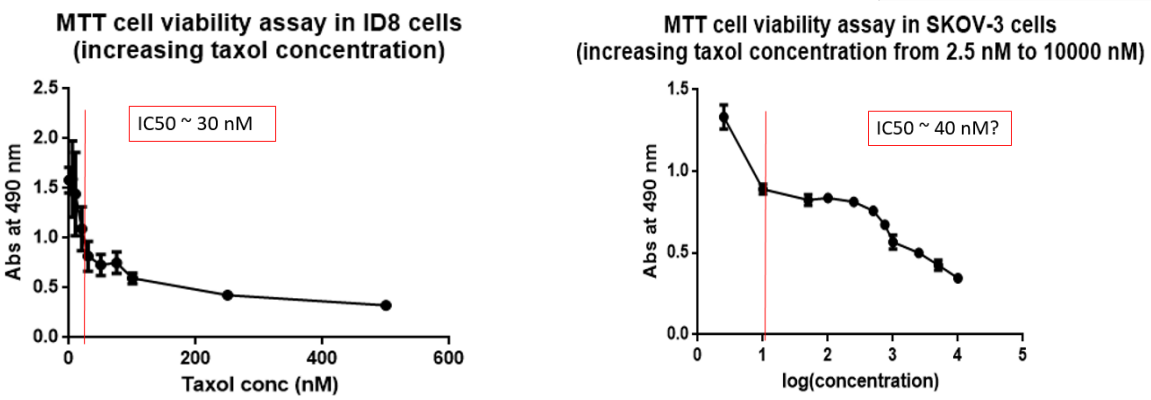
Aim 1, Subtask 2: Test ability of p53-CCmut with or without CPTX to inhibit cell proliferation and oncogenic potential (trypan blue, MTT, CFA) in cells with varying p53 status.

Major activities, specific objectives, results:

Determining estimates of IC50 values for paclitaxel and carboplatin in ovarian cancer cell lines: major activities and specific objectives are to determine 50% inhibitory concentrations of paclitaxel (taxol) and carboplatin in ovarian cancer cell lines. This was performed by adding increasing drug doses to cells, and measuring cell death using the MTT cell viability assay. IC50's for ID8 cells

were ~30nm, and for SKOV-3 cells, ~40nM (Figure 8). IC50's for other cell lines not tested due to data collected later showing issues with transfection and drug addition. Carboplatin was problematic since the first time we purchased it (Sigma), the drug was inactive (ie, killing was not occurring at even very high doses). Repurchasing from another vendor also led to the same results, unfortunately.

Figure 8. Determination of approximate IC50 in ID8 and SKOV-3 cells.



Combination studies with our gene constructs and taxol, with carboplatin, and with both drugs (CPTX): major activities and specific objectives are to determine if combination gene and drug treatment result in increased apoptosis of ovarian cancer cells *in vitro*. We transfected cells with our gene constructs, p53-CC and/or p53-CCmut (also known as p53-CC*). Gene constructs are first transfected into cells, then drug (taxol to start) is added, and the 7AAD apoptosis assay is performed 48h later. Kuramochi, ID8, and SKOV3 were the first cell lines tested as shown in Figures 9-11. For Figures 9 and 11, EGFP gated (green bars) and all cells (red bars) shown in graph; for Figure 10, only EGFP gated cells shown. The pilot data in these figures is fairly complex, and we note differences in effect between all 3 cell lines. Some trends can be gleaned from these pilot experiments. Note are that in Kuramochi cells, taxol displays a typical dose related effect, and

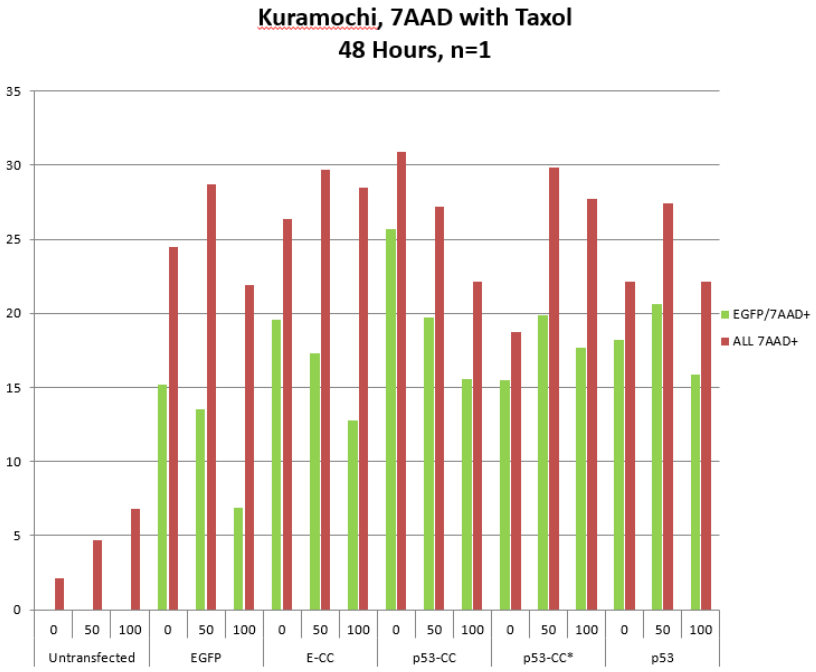


Figure 9. Kuramochi cells with various treatments. Untransfected = no gene transfected. EGFP, EGFP-CC, p53-CC, p53-CCmut (*), and p53 transfected cells were treated with 0, 50, or 100nM doses of taxol.

transfection may be contributing to cell death (regardless of what plasmid is transfected, Figure 6). In ID8 cells, the p53-CC (or p53) alone causes robust apoptosis, irrespective or taxol added (Figure 7). In Figure 8, it appears that taxol may "interfere" with transfections (and hence the ability of these constructs to cause apoptosis), so the timing of dosing must be optimized.

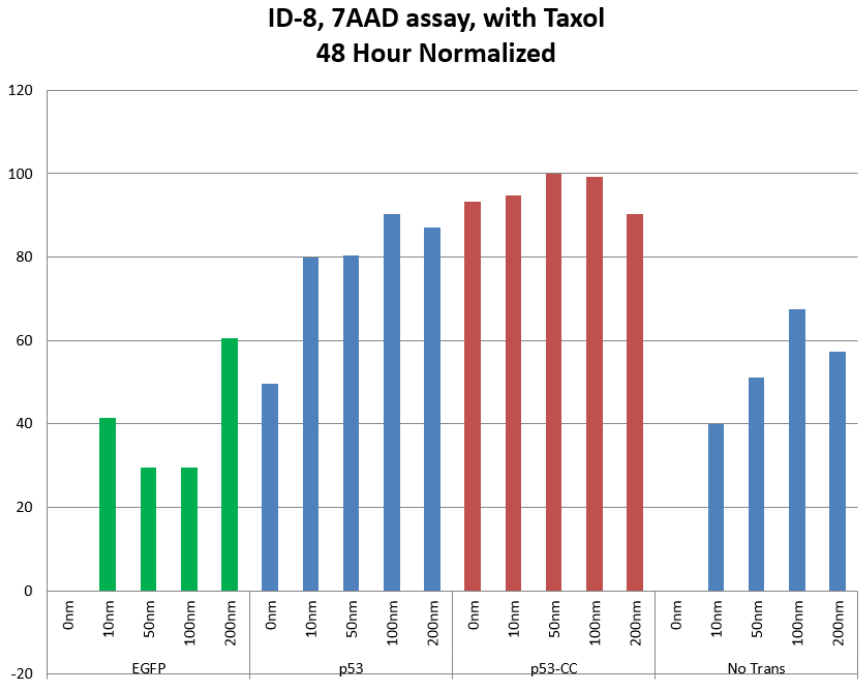


Figure 10. ID8 cells with various treatments. Untransfected = no gene transfected. EGFP, p53, or p53-CC transfected cells or untransfected cells were treated with 0, 50, or 100nM doses of taxol.

SKOV3 cells were grown in DMEM with 10% HI FBS, 1% penicillin/streptomycin/L-glutamine. For 7AAD assay, used 250,000 cells plated per 6 well plate, transfected 24h later using 5ug plasmid DNA and 5ul Lipofectamine 2000.

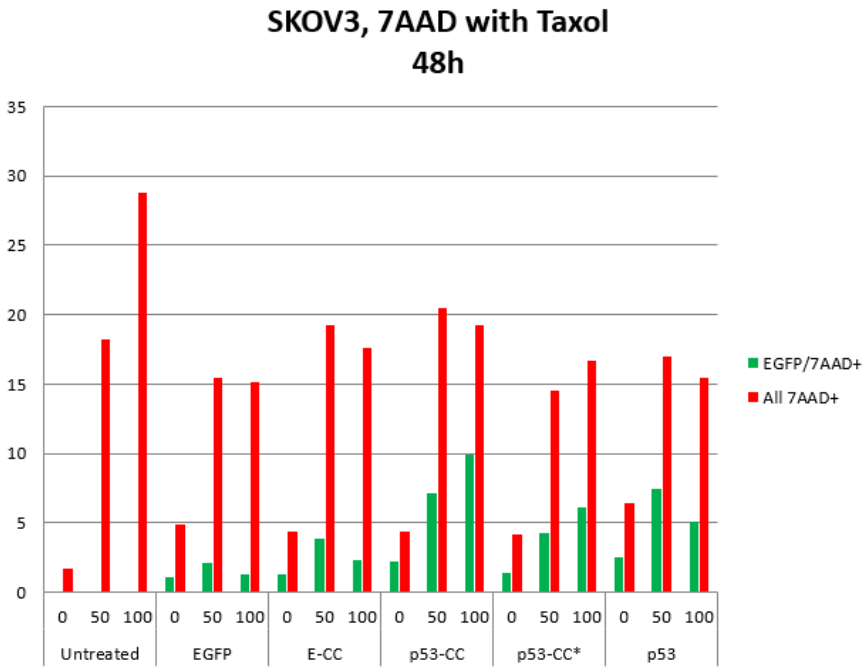


Figure 11. SKOV3 cells with various treatments. Untreated = no gene transfected, EGFP, EGFP-CC, p53-CC, p53-CCmut (*), or p53 transfected cells were treated with 0, 50, or 100nM doses of taxol.

A more complete combination study was attempted to look at the effect of paclitaxel on gene transfection (Fig. 12) using p53-Bax MTS with paclitaxel. Fig. 12 shows that using paclitaxel greatly reduces the number of gene-transfected cells, regardless of what gene is used (including GFP control). This indicates that attempting to do combination

therapy in vitro may be extremely difficult; it does not mean that combination therapy would be ineffective in vivo, though.

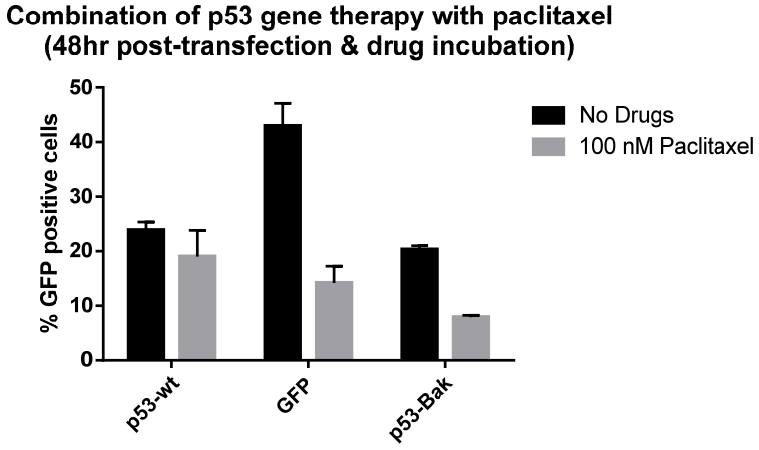


Figure 12. The effect of paclitaxel on transfection of SKOV3 cells. In general, paclitaxel reduced the transfection of SKOV-3 cells regardless of the gene transfected.

Aim 1, Subtask 1: deliver p53-CCmut plasmid with PEG-PEI-cholesterol (WSLP) polymer to ID8 ovarian cancer cells; measure transfection efficiency and potency.

While WSLP polymer was successfully synthesized by Dr. Nam (post doc in S.W. Kim's lab), transfections using WSLP were not successful (very low transfection efficiency, data not shown), so we proceeded to subtask 2.

Aim 1, Subtask 2: construct adenoviral vector containing p53-CCmut; deliver p53-CCmut (in adenovirus with CD-PEG-RGD) polymer to ID8 ovarian cancer cells; measure transfection efficiency and potency.

CD-PEG-RGD (bioreducible poly(cystaminebisacrylamide-diaminohexane)-polyethylene glycol linked to a RGD (Arg-Gly-Asp)) was successfully synthesized using published methods by Dr. Nam with PEG1000 (Mal-PEG-NHS 1K from Biochempeg) and cyclic RGD peptide from NovoPro Biosciences (cat #303458). Conjugation and size were verified by NMR. Adenoviral vectors (AdenoX System 3, Clontech) containing wt-p53, p53-CC, and ZsGreen (control) were made. Ad-ZsGreen was tested in ID8 cells, which showed very high expression (Fig. 13). For coating adenoviral vectors with polymer, polymer and adenovirus were simply mixed by tapping, and after 60 min at RT, added to cells in serum free media and incubated for 24h prior to measuring expression levels. A 0.3% (%RGD conjugation) and a 0.31×10^4 polymer:Ad ratio (using Ad-CMV-ZsGreen control) had optimal expression (%GFP positive) and minimal toxicity *in vitro* in ID8 cells (Fig. 13). It should be noted that the polymer was not expected to increase transfection efficiency in vitro (but in vivo, should minimize immunogenicity, and could increase transfection efficiency in the mouse model).

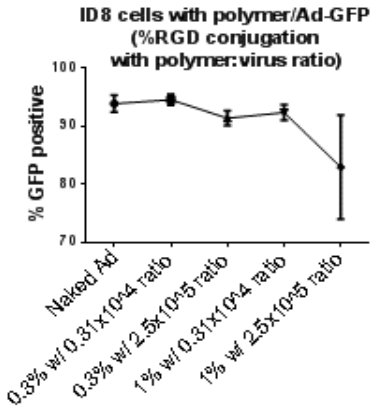


Figure 13. Polymer Optimization.

Aim 3, Subtask 1: establishing primary tumor within ovary.
In a pilot assay, 6-8 week old female C57BL/6 mice (Jackson Laboratories) had a single dorsal midline incision made to access ovaries. 1×10^6 ID8 cells were injected into left ovarian

bursa (PBS control injected into contralateral ovary), and monitored daily. Tumors were grown ~6-8 weeks (2-2.5cm^3), prior to initiating treatment (before mets and ascites form). 500ul volume IP injections [114, 115] of polymer-Ad (using 0.3% conjugation ratio, at 0.31x10^4 and 2.5x10^5 polymer:Ad ratios were given [116] once daily for 10 days. After completion of treatment, mice were weighed, sacrificed, organs harvested, and tumors excised (tumor volume and weight measured; ascites collected) as before. On a preliminary toxicity study using IP injected polymer alone (dashed line, n=2) and polymer with Ad (solid line, n=3) compared to PBS control (dotted line, n=2) in mice, after 10 days of injections, animals showed no gross toxicity/no weight loss (Fig. 14), and no gross immunogenicity (no swelling, inflammation, or redness at injection site; data not shown). Harvested ovaries indicate growth of tumors (left ovary with injected tumor cells, vs. untreated right ovary, Fig. 15).

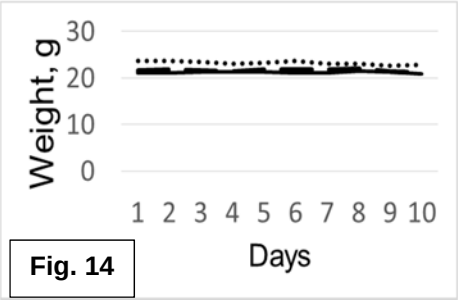


Fig. 14

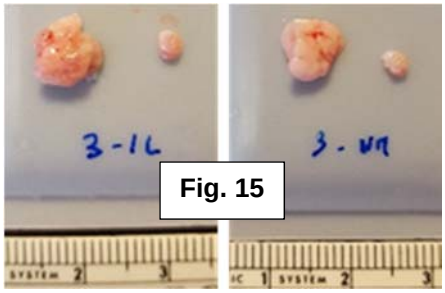


Fig. 15

Summary:

In this report, gene therapy using re-engineered super p53 (p53-CC constructs) was not as effective as we had hoped (not superior to wt p53) and showed toxicity in normal cells. However, mitochondrially targeted p53 constructs may be more potent in ovarian cancer cells. **Main activities and objectives** completed include optimization of cell culture growth and determining transfection conditions for human ovarian cancer cell lines, including Kuramochi, OVCAR3, SKOV3, SKOV3.ip1, and mouse ovarian cancer cells (ID8). 7AAD and MTT assay conditions have been optimized. WSLP (polymer) and CD-PEG-RGD polymers have been successfully synthesized, and a subset of adenoviral constructs have been cloned (p53, p53-CC, EGFP control). **Major results**: Gene therapeutic super p53 (p53-CC) localizes mainly to the nucleus in human ovarian cancer cells (Kuramochi) and exclusively in mouse cells (ID8) as demonstrated by fluorescence microscopy. Mitochondrially directed p53 localizes to the mitochondria, and causes robust apoptosis OVCAR-3 and ID8 cells, and may be our new lead construct. Combination treatment (gene and paclitaxel) proved to be difficult to achieve in vitro due to the lowering of the transfection efficiency of the gene when paclitaxel was used. If used in vivo, this could be overcome by staggered dosing of the 2 agents. The ovarian cancer animal model was successfully established, and we will continue to test our mitochondrial gene therapy in this model. We are very grateful for this funding from the DOD, as it has solidly established our lab in the area of ovarian cancer. This grant has provided invaluable training in ovarian cancer research for the PI, Co-I, and students. Our goal is to continue this work, and develop a therapeutic for ovarian cancer.

- **What opportunities for training and professional development has the project provided?**

- Nothing to report (Informally, grad student has presented data from this project in lab meeting multiple times, and the project has been discussed in terms of troubleshooting and direction. New lab members are being trained on the project, and students will present their work in departmental seminar and at the AACR Annual Meeting in Chicago, 2018).
- **How were the results disseminated to communities of interest?**
  - Nothing to report yet, but grad student has present work in departmental seminar and 2 students will report their findings at the AACR Annual Meeting in Chicago, 2018.
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - Nothing to report, although we are continuing with the experiments even without DOD funding.

4. **IMPACT:**
- **What was the impact on the development of the principal discipline(s) of the project?**
- Nothing to report yet, but when published/completed may provide a new gene therapeutic/combination therapy for ovarian cancer.
- **What was the impact on other disciplines?**
- Nothing to report.
- **What was the impact on technology transfer?**
  - Nothing to report.
- **What was the impact on society beyond science and technology?**
- Nothing to report yet; possibly a new therapy for ovarian cancer.

5. **CHANGES/PROBLEMS:**
- **Changes in approach and reasons for change**

  No changes in approach/objectives/scope. We did introduce a related p53 construct that was more potent than the proposed p53-CC.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - We were unable to hire a full time student at the onset of the project, but were able to recover and had 3 new graduate students and an undergraduate student work on this project.
- **Changes that had a significant impact on expenditures**
  - Delays in hiring staff shifted expenditures to the latter part.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - Nothing to report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*
- **Publications, conference papers, and presentations**

  Nothing to report. Planning on 2 presentations at AACR meeting.
  - **Journal publications.**
  - Nothing to report; plan on publishing 1 manuscript within a year.
  - **Books or other non-periodical, one-time publications.**
  - Nothing to report.
  - **Other publications, conference papers, and presentations.**
  - Nothing to report.

- **Website(s) or other Internet site(s)**
  Nothing to report.
- **Technologies or techniques**
  Nothing to report.
- **Inventions, patent applications, and/or licenses**
  Nothing to report.
- **Other Products**
  Nothing to report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	*Carol Lim*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1.5*
Contribution to Project:	*Lim has directed the entire project thus far, and has been involved with experimental design, troubleshooting, and analysis of data.*
Funding Support:	*National Institutes of Health, NCI*

Name:	*Margit Janat Amsbury*
Project Role:	*Key Collaborator, Co-I*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1.5*
Contribution to Project:	*Dr. Amsbury has provided assistance with cell lines, drugs, and planning experiments.*
Funding Support:	*National Institutes of Health, NCI*

Name:	*Benjamin Bruno*
Project Role:	*Graduate Student*

Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Bruno has designed, conducted, troubleshot, and analyzed the in vitro experiments in this proposal.*
Funding Support:	*n/a*

Name:	*Erica Van Der Mause*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*12*
Contribution to Project:	*Will design, conduct troubleshoot, and analyze the in vitro experiments in this proposal (training by Bruno)*
Funding Support:	*n/a*

Name:	*Katherine Redd*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Will design, conduct troubleshoot, and analyze the in vitro experiments in this proposal (training by Bruno)*
Funding Support:	*n/a*

Name:	*Matt Aspinwall*
Project Role:	*Undegraduate Student Technician*

Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1*
Contribution to Project:	*Will design, conduct troubleshoot, and analyze the in vitro experiments in this proposal (training by Bruno)*
Funding Support:	*n/a*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- A grant was awarded: NIH R21 CA187289-02 (07/07/2015 - 06/30/2017) with no changes in level of effort for this DOD grant. Lim is PI and Janat-Amsbury is Co-I.

- **What other organizations were involved as partners?**
  - Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** *not applicable*
   - **QUAD CHARTS:** *not applicable*

9. **APPENDICES:** *not applicable*

********** **ADDITIONAL NOTES:** not applicable